Serenade ASO
(*Bacillus amyloliquefaciens* QST 713)

Microbial pest control product against plant pathogenic fungi and bacteria

Dossier according to OECD guidance for industry data submissions for microbial pest control products and their microbial pest control agents – August 2006

Summary documentation, Tier II

Annex IIIM1, Section 6

Point IIIM1 11: Summary and evaluation of environmental impact

Date: September 2015

Revised November 2015

Applicant

Bayer CropScience AG

Table of contents

OWNERSHIP STATEMENT

Introduction

The company Bayer CropScience AG is submitting a dossier for the re-approval of *Bacillus amyloliquefaciens* QST 713, previously designated as *Bacillus subtilis* QST 713, as an active substance under regulation (EC) 1107/2009. Due to changes in taxonomy, *B. subtilis* QST 713 is now classified as *B. amyloliquefaciens*. For further information, please refer to Annex II, Section 1, Point IIM 1.3.1 of this dossier. As a consequence, the active substance is now named *B. amyloliquefaciens* QST 713. The old strain designation is still used in some documents and can be considered as a synonym. Serenade ASO is the representative formulation for the process of the re-approval of *Bacillus amyloliquefaciens* QST 713 as an active substance under regulation (EC) 1107/2009.

Inclusion of *B. subtilis* QST 713 into Annex I of 91/414/EEC (now list of approved active substances according to (EU) No 540/2011) entered into force in February 2007 (Commission Directive 2007/6/EC[1]). *B. subtilis* strain QST 713 was notified and defended by AgraQuest Inc. Although formulation Serenade ASO was not the representative formulation in the dossier for Annex I inclusion of *B. subtilis* QST 713, here the data of above mentioned product is summarized, since it represents latest information on *B. amyloliquefaciens* QST 713 formulation. The representative formulation for the initial Annex I inclusion, Serenade WP, is no longer produced.

Here we submit all studies reviewed on the zonal level and new data and information (public literature and summaries). The information for studies submitted on the zonal will appear in blue font. The test substance as it appears in the reference study will be used, new calculations will use the new strain designation.

Critical Good Agricultural Practices for Serenade ASO are summarized in the table below.

Table 10-1: Proposed use pattern of Serenade ASO (professional use)

Crop and/ or situation (crop destination / purpose of crop)	F G or I	Pests or Group of pests controlled	Application			Application rate			PHI (days)	Remarks
			Method / Kind	Timing / Growth stage of crop & season	Max. number (min. interval between applications) a) per use b) per crop/ season	L product / ha a) max. rate per appl. b) max. total rate per crop/season	kg as/ha a) max. rate per appl. b) max. total rate per crop/season	Water L/ha min / max		
Strawberry	G	*Botrytis cinerea*	Spraying	BBCH 55-89	a) 6 (5 days) b) 6 (5 days)	a) 10 b) 60	a) 0.140 kg min. 1x 10^{13} CFU/ha b) 0.84 kg min. 6 x 10^{13} CFU/ha	400-1000	n.r.	10 L/ha authorized in UK
Strawberry	F	*Botrytis cinerea*	Spraying	BBCH 55-89	a) 6 (5 days) b) 6 (5 days)	a) 8* b) 48	a) 0.112 kg min. 8 x 10^{12} CFU/ha b) 0.672 kg min. 4.8 x 10^{13} CFU/ha	400-1000	n.r.	
Grapes	F	*Botrytis cinerea*	Spraying	BBCH 68-89	a) 9 (5 days) b) 9 (5 days)	a) 8 b) 72	a) 0.112 kg min. 8 x 10^{12} CFU/ha b) 1.008 kg min. 7.2x 10^{13} CFU/ha	500-1000	n.r.	

n.r. – not relevant

* Please note for the purposes of calculating PEC values and risk assement the rate in Kg product/ha and CFU/g values were used as noted in the tables.

IIIM1 11 Summary and evaluation of environmental impact

All literature searches were conducted to include information for both the original active ingredient designation of *Bacillus subtilis* and *Bacillus amyloliquefaciens*.

IIIM1 11.1 Distribution and fate of MPCP

Fate and behaviour in soil

Based on available information derived from studies and published literature on *Bacillus subtilis* and *Bacillus amyloliquefaciens* bacterial strains, the environmental fate and population dynamics of *B. amyloliquefaciens* QST 713 upon field application of Serenade ASO can be summarized as follows:

Bacillus subtilis and *B. amyloliquefaciens* are both members of the natural micro-flora in soils and occur without geographical restriction. Following an application of Serenade ASO, survival of the endospores of *B. amyloliquefaciens* in soil is restricted to a period of a few months during which time a natural breakdown begins and gradually reduces the numbers of spores remaining. It is very unlikely that endospores of *B. amyloliquefaciens* will germinate and grow into vegetative cells, unless encouraging conditions exist, meaning favourable soil pH, soil moisture content, sufficient nutrient availability and lack of competition / predation from other soil micro-organisms.

Finally, introduced *B. amyloliquefaciens* cells and spores are not expected to exceed the natural level in soil permanently.

The highest predicted environmental concentration of Serenade ASO is 96 mg Serenade ASO/kg dry weight soil (1.34 mg *B. subtilis*/kg dry weight soil). In terms of CFU, this is equivalent to 9.6×10^7 CFU/kg dry weight soil.

Fate and behaviour in water

Surface water

Bacillus subtilis and *B. amyloliquefaciens* is frequently occurring in different aquatic environments, as fresh water, estuarine and coastal waters and endospores have been detected in sediments and even in the open ocean. However, *B. subtilis* and *B. amyloliquefaciens* does not find optimal conditions for growth, e.g. waters are poor in organic C. Therefore, proliferation is not likely to occur. It may be stated that *B. subtilis* and *B. amyloliquefaciens* is inactivated in water under natural conditions, including water.

Ground water

Bacillus subtilis and *B. amyloliquefaciens* endospores are reported to as having longevity in groundwater. However, *B. subtilis* and *B. amyloliquefaciens* do not find optimal conditions for growth, e.g. waters are poor in organic content. Therefore, proliferation in ground water is not likely to occur.

Considering the natural distribution of *B. subtilis* and *B. amyloliquefaciens*, as an integral part of the soil-microflora, no detrimental concern is attributable to field applications of the *B. amyloliquefaciens*, containing product Serenade ASO. Therefore only a negligible amount of *B. amyloliquefaciens* is expected to reach ground water. It is thus concluded that no threat of contamination of groundwater exists following applications of Serenade ASO according to GAP.

Fate and behaviour in air

Endospores are suitable for aerial distribution as they are easily blown about by wind. Therefore, under conditions of use drift spacious transport may occur. Multiplication of *B. subtilis* and *B. amyloliquefaciens* in the air, aerosols or clouds can be excluded due to lack of organic matter supply and lack of mineral matrix to adhere to.

Furthermore, unlike chemical products, evaporation and volatility of bacteria is not expected to be a factor to consider in assessing the fate in air. Hence volatilisation from plant surfaces and from soil can be excluded. In addition, in air *B. subtilis* and *B. amyloliquefaciens* cells are exposed to several environmental stress factors (desiccation, UV-radiation, temperature). Therefore, survival of vegetative cells in air is limited.

A summary of PEC_{Soil} and PEC_{SW} calculation is presented in **Tables IIIM1 11.1-1** and **IIIM1 111.1-2,** respectively.

The calculation was based on the accumulated field rate of Serenade ASO in grapes, with a maximum of 9 applications.

Due to the PEC_{SW} calculation, the initial concentration of Serenade ASO in 30 cm depth in surface waters is 1502.4 µg/L (21.03 µg *B. amyloliquefaciens*/L) corresponding to 1.5×10^6 CFU/L.

Table IIIM1 11.1-1 Summary of PEC_{Soil} calculations

Critical use	Grapes, maximum of nine applications with 8 Kg Serenade ASO/ha each
Accumulated application rate	72 kg Serenade ASO/ha, 1.008 kg *B. amyloliquefaciens* QST 713/ha, 7.2×10^{13} CFU/ha
Soil density	1.5 g/cm³ (= 75 kg soil/ m²)
Incorporation depth	5 cm layer (= 50 L soil/m²)
Plant interception	Not considered
PEC_{Soil}	96 mg Serenade ASO/kg dry weight soil, 1.34 mg *B. amyloliquefaciens* QST 713/kg dry weight soil, 9.6×10^7 CFU/kg dry weight soil

Table IIIM1 11.1-2 Calculation of the predicted environmental concentration of Serenade ASO in lentic water bodies (PEC_{SW})

Application rate kg/ha	Rate mg/m²	Distance (m)	Drift (%)[b]	Amount of drift		Initial PEC_{SW} [µg/L]	
				g/ha	mg/m²	1 m	30 cm
72[a]	7200	3	6.26	4507.2	450.7	450.7	1502.4[c]

[a] Accumulated application rate of Serenade ASO for GAP directed use in grapes 9 × 8 kg/ha)
[b] According to Julius Kühn Institut[1], status September 2015.
[c] Equivalent to 1.5×10^6 CFU/L or 21.03 µg *B. amyloliquefaciens* QST 713/L

IIIM1 11.2 Identification of non-target species at risk and extent of their exposure

According to the presented risk assessment, the use of Serenade ASO at the proposed label rates according to good agricultural practice poses no risk to any of the non-target species.

Effects on birds

Birds are not considered to be at risk upon application of Serenade ASO. This was confirmed by the absence of treatment related mortalities or effects upon oral administration in birds and the TER_A values for grapes and strawberries (following First Tier assessment) exceed the Annex VI trigger value of 10, indicating that Serenade ASO poses no risk to birds following application according to the proposed use patterns of these crop scenarios.

Effects on fish

The long-term TER value of fish for Serenade ASO exceeds the Annex VI trigger value of 10 indicating that no adverse effects are to be expected upon field application at recommended use levels. Due to the absence of toxicity in the semi-static studies conducted over a period of 30 days no acute risk for fish is expected upon short term exposure to Serenade ASO**.**

Effects on freshwater invertebrates

The acute TER value for daphnids is are above the Annex VI trigger of 100, indicating a low acute risk to *D. magna* following GAP directed application of Serenade ASO. Considering the absence of acute toxicity and the NOEC obtained in the 21-day semi-static tests (1.5×10^8 CFU/L) that is more than 10-fold higher than the PEC_{SW} (1.56×10^6 CFU/L) no adverse effects on daphnids are to be

[1] Basic Drift Values according to Julius Kühn Institut: status September 2015, http://www.jki.bund.de/no_cache/en/startseite/institute/anwendungstechnik/abdrift-eckwerte.html

expected even upon prolonged exposure to serenade ASO. Prolonged exposure, however, is not likely to occur due to the restricted persistence of *B. amyloliquefaciens* QST 713 in water.

Effects on single cell algae

The long-term TER value of algae for *B. amyloliquefaciens* QST 713 strongly exceeds the Annex VI trigger value of 10 suggesting that no negative side effect is expected following field application according to GAP.

Effects on aquatic plants other than algae

The long-term TER value of algae for *B. amyloliquefaciens* QST 713 strongly exceeds the Annex VI trigger value of 10 suggesting that no negative side effect is expected following field application according to GAP.

Effects on terrestrial plants

No information is provided however as the active ingredient is not a plant pathogen no adverse effects on terrestrial plants are expected when the product is used according to label instructions.

Effects on terrestrial vertebrates other than birds

Acute and short-term toxicity studies with *B. subtilis* QST 713 containing products in rats confirm the absence of toxicity to mammals. Using the highest LD_{50} value of *B. subtilis* QST 713 obtained during acute oral toxicity studies for a risk assessment, the calculated TER values, including those for critical uses, indicate that no unacceptable risk is to be expected for mammals upon field application of Serenade ASO according to GAP.

Effects on bees

From the results of all studies it can be concluded, that application of Serenade ASO according to Good Agricultural Practice intended uses, does not pose a risk to honey bees.

Effects on arthropods other than bees

Following the result of the non-target arthropod risk assessment the HQ values for the in-crop scenarios as well as for the off-crop scenarios are below the trigger of 2, the ESCORT 2 document and SANCO/10329/20023 demand no further higher tier testing. No unacceptable risk is to be expected upon field application of Serenade ASO according to GAP.

Effects on earthworms

The acute TER value of earthworms for Serenade ASO exceeds the Annex VI trigger value of 10 indicating that GAP directed application of Serenade ASO poses no acute risk to earthworms.

Effects on soil micro-organisms

The ingredients of the preparation Serenade ASO, formulated as a suspension concentrate, are inert, non-toxic and impose no environmental or health risk. Literature on possible effects of *B. subtilis* and *B. amyloliquefaciens* on soil microflora shows that its introduction to soil does affect soil microbial activity.

The *B. amyloliquefaciens* and *Bacillus subtilis* are members of the natural micro-flora in soils worldwide. Therefore, its possible multiplication in this natural habitat does not disturb the natural micro-flora. As vegetative growth declines with declining nutrient source this species does not seem to compete well for limited resources and *B. subtilis* and *B. amyloliquefaciens* populations will be subject to competition in the natural micro-flora on ecological basics.

In conclusion, negative effect to the soil microflora following application of Serenade ASO according to GAP directed uses are not expected.

IIIM1 11.3 Identification of precautions necessary to minimize environmental contamination and to protect non-target species

The risk assessment proves that Serenade ASO is not toxic to the tested aquatic and terrestrial species, and considering the expected environmental concentration will not be hazardous to natural populations upon applications according to Good Agricultural Practice. No hazard classification or specific labelling according to EC Directive 67/548/EEC is required for Serenade ASO.